FUNDAMENTAL STUDY OF PULSE ELECTRIC FIELD EFFECTS ON HELA CELL CULTURED OVER EXTRACELLULAR MATRIX PROTEIN MICRO-PATTERNED SURFACE

NUR ADILAH BINTI ABD RAHMAN

A thesis submitted in
fulfilment of the requirement for the award of the
Degree of Master in Electrical Engineering

Faculty of Electrical and Electronic Engineering
Universiti Tun Hussein Onn Malaysia

AUGUST 2017

For my beloved Mother and Father

ACKNOWLEDGEMENT

I would like to take this opportunity to express my greatest gratitude to almighty Allah for His help and support during the course of life and the moment of truth.

I would like to express my appreciation and sincere gratitude to my supervisor, Associate Professor Dr. Muhammad Mahadi Bin Abdul Jamil for his continuous support, encouragement and endless patience towards completing the course of this master research. I am thankful for his aspiring guidance, invaluable construction criticism, and friendly advice during the project work. I am sincerely grateful to him for sharing his truthful and illuminating views on a number of issues related to the project.

I would like to express my appreciation to both of my beloved parents, my dad, Hj. Abd Rahman B. Abd Samat and my mom, Hjh. Rohaya Bt. Hashim for their tremendous patience, effort, support and encouragement during my master research. I really appreciate it. I would also sincerely like to thank my friends, Noor Hafizzatul Izzah Bt. Mat Harun, Nur Shazilah Bt. Aziz and Atikah Bt. Daud, for their support, encouragement and advice during my master and also to all the people who have contributed directly or indirectly for the accomplishment of this master research.

I would like to express my appreciation to UTHM, for the sponsor of my studies via GIPS (Vote: U167) until the completion of my studies. Last but not least, the Ministry of Higher Education for the sponsor of research materials and conference travel fund through Fundamental Research Grant Scheme (FRGS Vote: 1488). Thank you.

ABSTRACT

Electroporation (EP) is a method of controlling cell function by using pulses of electrical fields to create pores through cell membrane and causes other substance around it to be absorbed into the cell. This method has led to a variety of medical applications, particularly in cell studies. In this study, a high voltage of 2 kV/cm with pulse duration of 30 µs was applied on HeLa cell (human cervical cancer cell) to investigate the electroporation process. In addition, this study focused on the effect of protein coated surface, combined with the pulse parameter mentioned above, to look at its effect on HeLa cell when exposed to high voltage. Thus, will lead towards cell surface attachment factors interrogation plus the presence of electric field as the stimulator for an aggressive growth rate of the cells. This was achieved by using the micro contact printing (µCP) method. The result showed positive respond on the effect of EP on protein printed surface combination where HeLa cells were grown. The 50µm was chosen as the best-pattern size for cell alignment by using fibronectin. From the cell guidance study we could clearly see the cell responses on the protein patterned surface are much elongated in comparison to the control. In addition, the cells plated on this patterned surface were further investigated with electroporation technique, in order to see the effect of electroporation on the cancer cell proliferation and other cellular activities. The result shows that the cells aligned and elongated on fibronectin pattern with PEF than without PEF exposure. The combination of these two techniques will contribute towards understanding the cell surface interface and cell surface attachment factors which may lead towards a new method for guiding cell towards wound healing process.

ABSTRAK

Electroporation (EP) merupakan satu kaedah yang digunakan untuk mengawal fungsi sel dengan menggunakan denyutan medan elektrik bagi mewujudkan liang pada permukaan sel membran dan menyebabkan molekul lain yang berada di sekelilingnya diserap masuk ke dalam sel. Kaedah ini telah membawa kepada pelbagai aplikasi perubatan, terutamanya dalam kajian sel. Dalam kajian ini, voltan tinggi 2 kV / cm dengan tempoh denyutan 30 μs digunakan pada HeLa sel (sel kanser pangkal rahim) untuk mengkaji proses EP. Selain daripada itu, kajian ini memberi tumpuan kepada kesan permukaan bersalut protein digabungkan dengan parameter EP yang dinyatakan di atas, untuk melihat kesan ke atas sel HeLa apabila terdedah dengan voltan tinggi. Oleh itu, akan membawa ke arah faktor lekatan sel pada permukaan kajian ini ditambah dengan kehadiran medan elektrik sebagai perangsang untuk meningkatkan kadar pertumbuhan sel. Ianya dicapai dengan menggunakan kaedah *microcontact printing* (μCP). Hasil kajian menunjukkan tindak balas positif pada kombinasi kesan EP pada permukaan protein bercetak di mana sel HeLa telah diletakkan. Saiz 50 μm dipilih kerana saiz corak terbaik bagi penjajaran sel dengan menggunakan fibronectin. Daripada kajian bimbingan sel ini kita dapat melihat dengan jelas tindak balas sel pada permukaan protein bercorak dengan lebih memanjang berbanding dengan kawalan. Di samping itu, sel yang diletakkan pada permukaan bercorak ini telah dikaji pula dengan teknik EP, bagi melihat kesan EP pada percambahan sel kanser dan aktiviti sel lain. Hasil kajian telah menunjukkan bahawa sel sejajar dan memanjang pada permukaan *fibronectin* bercorak di bawah pendedahan PEF berbanding tanpa pendedahan PEF. Gabungan kedua-dua teknik ini akan menyumbang ke arah pemahaman antara interaksi permukaan sel dan faktor lekatan permukaan sel yang boleh membawa ke arah satu kaedah baru untuk membimbing sel ke arah proses penyembuhan luka.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS AND ABBREVIATIONS

µCP	Micro contact printing
CAD	Computer Aid Design
DNA	Deoxyribonucleic acid
EC magnetic Chamber	Perfusion type electrical stimulation magnetic chamber
ECM	Extracellular Matrix
EP	Electroporation
FBS	Fetal Bovine Serum Protein
FBS	Fetal Bovine Serum
FN	Fibronectin
PBS	Phosphate Buffered Saline
PDMS	Polydimenthylsiloxane
PEF	Pulsed electric field
Pen/Strep	Penicillin-streptomycin
RNA	Ribonucleic acid

SAMs	Self-Assembled Monolayers
TC	Type of Chambers
GAGs	Glycosaminoglycans

LIST OF PUBLICATION AND AWARDS

Journal:

1. **Nur Adilah Abd Rahman**, Mamman Hassan Buhari, and M. Mahadi Abdul Jamil, "An Overview: Investigation of Electroporation technique on cell properties cultured on Micropatterned surface." Jurnal Teknologi, Vol. 77, No. 6, Medical Engineering Vol. 1, Pg. 61-65, 2015.

2. Safyzan Salim, **Nur Adilah Binti Abd Rahman**, M. Mahadi Abdul Jamil, Mansour Youseffi, Morgan Clive Thomas Denyer, "Investigation of Electroporation Technique On Cell Properties Cultured On Self Assembled Monolayer.", Journal of Biological Sciences, Vol. 16, No.7, Pg.278-283, 2016.

Book Chapter:

3. Muhammad A. Milad Zaltum, **Nur Adilah Abd Rahman** and M. Mahadi Abdul Jamil (2016). Pulse Duration Effect on Growth Rate of HeLa Cells. Muhammad Mahadi Abdul Jamil. Biomedical Engineering Applications: Cell Engineering, Penerbit UTHM, 4; 47-56.

Conference Proceedings:

4. **Nur Adilah Abd Rahman**, M. Mahadi Abdul Jamil, "Investigation of Pulsed Electric field on cancer cell cultured on patterned surface." IEEE, International Conference on control System Computing and Engineering (ICCSCE, 2016), 25th – 27th November 2016, Batu Feringghi, Pulau Pinang, Malaysia.

5. **Nur Adilah Abd Rahman**, M. Mahadi Abdul Jamil, "Enhancement of cell migration on protein pattern surface with the assistance of Pulsed Electric field: Cell Guidance Study." ASIA International Multidisciplinary Conference (AIMC-2017), 1-2 May, Universiti Teknologi Malaysia, Johor Bahru, Malaysia, 2017**. (Presented)**

6. M. Mahadi Abdul Jamil, **Nur Adilah Abd Rahman**, "Enhancement of cell migration and proliferation rate with the assistance of Pulse Electric field: Cell Migration Study.", International conference on electrical and electronic engineering 2017 (ICE3-2017), 8-9 May, Pulau Langkawi, Malaysia, 2017. **(Presented)**

Awards:

7. Investigation of Electroporation Technique On Cell Properties Cultured On Self Assembled Monolayer. Safyzan Salim, **Nur Adilah Binti Abd Rahman**, M. Mahadi Abdul Jamil, Mansour Youseffi, Morgan Clive Thomas Denyer. The International Conference on Engineering Technologies & Entrepreneurship 2015 (ICETE-2015), 16th - 18th November 2015, Kuala Lumpur, Malaysia.

   ("BEST PAPER AWARD")

Research Grant Award:

8. Grant: Investigation of Electroporation Effect on Cell Properties Cultured on Micropatterned Surface. Geran Insentif Penyelidikan Siswazah (GIPS), 31st March 2016.

CHAPTER 1

INTRODUCTION

1.1 Background of study

Micro contact printing (μCP) has been developed since about 20 years ago. It is an impressive surface patterning technique in micron and nano scale. Surface science communities such as engineers and biologists have been focusing in μCP and therefore enriching the improvement of the μCP process itself. A μCP is a soft lithography that is used for the release of pattern on master polydimenthylsiloxane (PDMS) stamp to form patterns of self-assembled monolayers (SAMs) print on the surface of a substrate by conformal contact. In the original version of μCP, the micrometre-scale patterned for chemical modification of a large surface area was found by transferring different types of compounds using soft polymer stamp (Maksud M. I., Yusof M. S., and Abdul Jamil M. M., 2013). The capability to generate patterns of proteins and cells on surfaces are important for biosensor technology (Thomas C. A., 1972, Gross G. W. et al., 1995, Jun D. R. et al., 1998 and Mrksich M. and Whitesides G. M., 1995), for tissue engineering (Merritt M. V., Mrksich M. and Whitesides G. M., et al., 1997) and for fundamental studies of cell biology (Mrksich M. et al., 1996b, Singhvi. R. et al., 1994, and Chen C. S. et al., 1997). Tissue engineering is essential for the cells to be placed in specific positions to create organized structures or it called cell migration which is important in tissue formation and cell guidance. Thus, the placement of biological ligands at well-defined locations on substrates is required for certain

biological assays (Ravi S. K. et al., 1999, R. Singhvi R. et al., 1994, Chen C. S. et al., 1997, Lopez G. P. et al., 1993 and Mrksich M. et al., 1996b).

Patterning technique controls the size and shape of the cell that is attached to a surface, the chemistry and the topology of the substrate to which the cell is attached towards cell guidance. The technique is also particularly useful in understanding the effect of cell-material interface on the behaviour of cells (Singhvi. R. et al., 1994, Chen C. S. et al., 1997, Lopez G. P. et al., 1993, and Mrksich M. et al., 1996). Photolithography is a technique that has been used widely for patterning proteins and cells (Ravi S. K. et al., 1999). It can be used to produce patterns by photo ablating proteins preadsorbed to a silicon or glass substrate (Hammarback J.A. et al., 1985). In order to see the patterned samples using inverted microscope, transparent material such as glasses is used as a substrate. Polydimethylsiloxane (PDMS) is suitable for patterning on glasses because it shows a good adhesion on glasses and it can be peeled off smoothly from the surface (Kyoko A. et al., 2004). There are several methods on controlling wound repair and cell behaviour by using a mixture of topographic guidance and topographic/adhesive guidance signals such as micro contact printing techniques (Abdul Jamil M. M. et al., 2007).

Electroporation (EP) or electropermeabilization is a method to introduce molecules, or a method for increasing cell membrane permeability to molecules by applying high magnitude electric pulses (Chunlan J. et al., 2015). It has been used in several biotechnological and biomedical applications, such as the introduction of molecules into cells, cell fusion, tissue ablation, and also sterilization of water and liquid food. (Davalos R. V. et al., 2004). This method can be applied in 3 ways that is *ex vivo, in vivo*, and *in vitro* (Chunlan J. et al., 2015). Cell electroporation *in vitro* is used mostly for transfection by DNA introduction and microbial killing. *Ex vivo* electroporation provides the influence of cells that is reintroduced into the body to provide therapy. *In vivo* electroporation of tissues boosts molecular transportation through the tissues and into their constitutive cells (Weaver J. C., 2000). By applying an electrical pulse across cells, a variety of outcomes can be obtained ranging from no effect to a reversible electroporation which transiently permeablize cell membrane (Zaltum M. A. M., Adon M. N. and Abdul Jamil M. M., 2013) to irreversible electroporation (Sundararajan R. et al., 2014). This technique has become a widespread technique for loading cell with substances because it can be implement to

any cell type (Zaltum M. A. M., Adon M. N. and Abdul Jamil M. M., 2013, Dev S. B. et al., 2000).

Electrically induced transfer of material into cells and tissues present an opportunity for many new medical treatments and provide a valuable tool for the study of the basic structural and biochemical behaviour of cellular and intercellular system (David W. J. et al., 2004). This technique has been found to be an effective technique to overcome membrane barrier (Dev S. B. et al., 2000). Briefly, if a membrane with a high electrical resistivity surrounds a biological entity with a low resistivity, as in the case of a cell, an applied electrical field is enhanced dramatically in the membrane thickness. Consequently, the high field strength in membrane can lead to the formation of area with increased permeability, or better known as pores, which allows transmembrane transportation of molecules (Dev S. B. et al., 2000). Furthermore, if the pulse electric field applied is not too strong and the exposure set is not too long, the pores reseal in seconds to minutes after the exposure and the cells return to their normal activity (Lea R. et al., 2013). Electric field does not only affect excitable tissue such as muscle and nerves but also the non-excitable tissue, either thermally, by producing heat inside the tissue or inducing structural changes down to cellular membranes (Zaltum M. A. M., Adon M. N. and Abdul Jamil M. M., 2013).

1.2 Problem statement

Wound healing is a process of replacing devitalized and absent cellular structure and tissue layer. For a human adult, wound healing process is divided into 3 phases which are inflammatory, proliferative and remodelling. However, in certain cases, there are problems regarding wound healing due to age related factors such as inability to form a blood clot, poor inflammatory response, inability to produce new cells, regeneration of new tissue and infection. These problems were tackled by using protein, drugs and cytokines such as transformation growth factor. This could be costly and very much chemical therapeutic compounds dependent.

Micro contact printing is a quite useful method in several applications such as extracellular matrix patterning for cell adhesion molecule to promote cell attachment

for the cell assembly, growth and cell guidance. It is important for the cell to be directed towards specific locations in order to enhance the wound coverage and thus resulting in tissue regeneration. The patterning technique can control both the size and shape of the cell that attaches to the surface of the substrate. Therefore, in this research, the micro patterning technique is combined with the electroporation method in order to look at the feasibility of pulsed electric field effect in enhancing the growth rate of cell. The combination also demonstrates the effect on the guidance of cell potentially as an alternative to the usage of readily available pharmaceutical chemical drugs and compounds.

1.3 Aim

The aim of this research is to study the effect of electroporation on HeLa cell properties cultured on ECM protein coated surface.

1.4 Objectives

This study embarks on the following objectives:

a) To investigate the electroporation effect on HeLa cell cultured on Self Assembled Monolayer and micro fabricated ECM protein surface.
b) To analyse the growth, proliferation rate, and alignment of the HeLa cell cultured on the two different surfaces mentioned.
c) To analyse the cell attachment factor on 2 different type of protein (fibronectin and fetal bovine serum).

1.5 Scopes of study

In order to achieve the objectives of this research, the following will be the scopes of work identified:

a) To use the PDMS stamp for patterning protein.
b) To culture and grow HeLa cells on self-assembled surface
c) To culture and grow HeLa cells on the micro patterned surface
d) To expose the plated cells with microsecond pulse via high voltage pulse generator with 2 kV/cm voltage, 30 µs pulsed length and single number of pulse.

CHAPTER 2

LITERATURE REVIEW

2.1 Electroporation

Electroporation or electropermeabilization, is a phenomenon where cell membrane permeability is increased to ions and macromolecules by exposing the cell to short high voltage field pulses (Ivorra A. and Rubinsky B., 2006). It is also called a viable technique where, a short duration pulses are applied to temporarily open up the pores on the cell membrane. This is due to the field enhancement in the plasma membrane of cells that allow transporting or introducing of therapeutic materials including drugs, antibodies, genes (DNA) and RNA as shown on Figure 2.1 (Mark J. J. et al., 1999, Sundararajan R. et al., 2012a, Rodamporn S., 2012 and Chunlan J. et al., 2015) which otherwise are impermeable (R. Sundarajan et al., 2011). In the cell electroporation, the applied electric field intensity influences the cell viability (Min-Ji K., Taeyoon K. and Young-Ho C., 2011). In comparison with other methods of gene transfer, electroporation is a non-invasive and nonchemical method. This method does not change the biological structure and function of the target cell itself (Rodamporn S., 2012).

REFERENCES

Abdul Jamil, M. M., Youseffi, M., Britland, S. T., Liu, S., See, C. W., Somekh, M. G. & Denyer, M. C. T. (2007). *Wide field Surface Plasmon Resonance Microscope: A Novel Biosensor Study of Cell Attachment to Micro patterned Substrates*. Springer Berlin Heidelberg, 3rd Kuala Lumpur International *Conference on Biomedical Engineering*, Kuala Lumpur, pp. 334-337.

Abdul Jamil, M. M. (2007). *Application of A Novel High Resolution Wide field Surface Plasmon Microscope in Cell Engineering, Wound Healing and Development of New Binding Assays*. Thesis Ph. D. University of Bradford.

Abraham, U. (1996). *Formation and structure of self-assembled monolayer*. Chem. Rev., 96:1533-1554.

Adon, M. N. (2015). *Pulsed Electric Field Exposure Effect on Morphological properties of HeLa cells*. Thesis Ph. D. Universiti Tun Hussein Onn Malaysia.

Akbulut, O., Yu, A. A., & Stellaci, F., (2010). *Fabrication of biomolecular devices via supramolecular contact based approaches*. Chem. Soc. Rev. 39, 30-37.

Alberts, B., Johnson, A., Lewis, J., et al. (2002). *Integrins*. Molecular Biology of the Cell. 4th edition. New York: Garland Science.

Alberts, B., Johnson, A., Lewis, J., et al. (2002). *The Extracellular Matrix of Animals*. Molecular Biology of the Cell. 4th edition. New York: Garland Science.

Andre, F., & Mir, L. M. (2004). *DNA electro transfer: its principles and an updated review of its therapeutic applications*. Gene Therapy. Therapeutics applications of DNA electro transfer. 11:33-42.

Anita, G., Mir, L. M., & Gehl, J., (2003). *Electrochemotherapy: results of cancer treatment using enhanced delivery of bleomycin by electroporation.* Cancer treatment reviews, anti-tumour treatment, 23:371-387.

Aune, T. E. V. & Aachmann, F. L. (2010). *Methodologies to increase the transformation efficiencies and the range of bacteria that can be transformed*. Appl Microbiol Biotechnol 85: 1301-1313.

Azioune, A., Carpi, N., Tseng, Q., Thery, M. & Piel, M. (2010). *Protein Micro patterns: A Direct Printing Protocol Using Deep UVs*. *Method in Cell Biology,* 8(97).

Banerjee, P., Mehta A. & Shanti, C. (2014). *Investigation into the cyto-protective and wound healing properties of cryptic peptides from bovine Achilles tendon collagen*. Journal of Chemico-Biological Interactions, 211:1-10.

Chen, C. S., Mrksich, M., Huang, S., Whitesides, G. M., & Ingber, D. E. (1997). *Geometric control of cell life and death.* Science, 279:1425-8.

Christian, F., Kathleen, M. S. & Valerie, M. W. (2010). *The extracellular matrix at a glance*. Journal of Cell Science, 123: 4195-4200.

Christophe, Y. C., Jessie, T., Christelle, L., Elodie, P., Thomas, B., Thierry, H., Pierre, L. D., & Lluis, M. M. (2014). *Optimization of a gene electro transfer procedure for efficient intradermal immunization with an hTERT-based DNA vaccine in mice*. Molecular Therapy, Methods and Clinical Development 1:14045.

Chunlan, J., Rafael, V. D., & John, C. B. (2015). *A review of basic to clinical studies of irreversible electroporation therapy*, IEEE transaction on biomedical engineering, 62:1.

Clark, P., Connolly, P. & Moores, G. R. (1992). *Cell guidance by micro patterned adhesiveness in vitro*. Journal of Cell Science, 103:287-292.

Curtis, A. & Riehle, M. (2001). *Tissue engineering: the biophysical background*. Phy. Med. Biol., 46(4):47-65.

Davalos, R. V. Mir, L. M. & Rubinsky, B. (2005). *Tissue ablation with irreversible electroporation*. Ann Biomed Eng, 33: 223-231.

Davalos, R. V., Otten, D. M., Mir, L. M. & Rubinsky, B. (2004). *Electrical Impedance Tomography for eImaging Tissue Electroporation*. IEEE Transaction on biomedical engineering, 51(5).

David, W. J., Ronald, M. G., Micheal, D. U., Linda, H. G. & Yue, Y. L. (2004). *Effect of pulsed high power radio frequency radiation on electroporation of mammalian cells*, IEEE transaction on plasma science, 32:4, 1573-1578.

DeLong, S. A., Gobin, A. S. & West, J. L. (2005). *Covalent immobilization of RGDS on Hydrogel cell alignment and migration*. Journal of Controlled Release 109, 139-148.

Dev, S. B., Rabussay, D. P., Widera, G. & Hofmann, G. A. (2000). *Medical Applications of Electroporation*. IEEE Transactions On Plasma Science, 28(1).

Dong, Q., Xia, Y. & Whitesides, G. M. (2010). *Soft lithography for micro- and nanoscale patterning*. Nature Protocols, 5(3): 36-39.

Folch, A. & Toner, M. (2000). Micro engineering of cellular interactions. Annu. Rev. Biomed. Eng., 2:227-56.

Gan, Y., Haiyam, L., Jiang, W. & Hua, H. (2008). *A novel bioreactor for wound healing study*. International conference on biomedical engineering and informatics.

George, D. O. (2014). *A multi-scale feedback control system model for wound healing electrical activity: Therapeutic device/protocol implications*. 36th Annual International Conference of the Engineering in Medicine and Biology Society (EMBC), IEEE, 26-30 Aug. 2014.

Gross, G. W., Rhoades, B. K., Azzazy, H. M. E. & Wu, M. C. (1995). *The use of neuronal networks on multielectrode arrays as biosensors*. Biosensor Bioelectric, 10:553-7.

Guan, G., Bai, L., Zuo, B., Li, M., Wu, Z. & Li, Y. (2009). *Scaffolds Decorated by in vivo Environment Improve Cell Proliferation and Wound Healing. Biomedical Engineering and Informatics*. *BMEI '09, 2nd International Conference* on 17-19 Oct. 2009.

Hammarback, J. A., Palm, S. L., Furcht, L. T., & Letourneau, P. C. (1985). *Guidance of neurite outgrowth by pathways of substratum - absorbed laminin*. Journal of neuroscience research, 13:213-220.

Hug, A., Jason, W., Nichol, C. B., Hutson, Hojae, B., Alisha, L. S., Cropek, D. M., Akhyari, P. & Khademhosseini, A. (2010). *Directed 3D cell alignment and elongation in micro engineered hydrogels*. Elsevier Biomaterials 31:6941-6951.

Imbeau, J., & Thouin, S. (1999). *What is the extracellular matrix (ECM)*? Integrative centre, Addison Wesley Longman, Inc.

Ivorra, A. & Rubinsky, B. (2006). *Impedance analyser for in vivo electroporation studies. Proceedings of the 28th EMBS Annual International Conference*, New York City, USA, Aug 30-Sept 3, 2006.

Johansson, F., Jonsson, M., Alm, K. & Kanje, M. (2010). *Cell Guidance by Magnetic nanowires*. Elsevier, Experimental cell research, 316; 688-694.

Joung, H. O., Hye, J. K., Tae, I. K. & Kyung, M. W., (2014). *Comparative evaluation of the biological properties of fibrin for bone regeneration*. The Korean Society for Biochemistry and Molecular Biology. 47(2). 110-114.

Jun, D. R. et al, (1998). *Cell Based microelectrode array characterized by imaging X-ray photoelectron spectroscopy, scanning electron microscopy, impedance measurements and extracellular recordings*. J Vac Sci Technology A, 16:1183-8.

Koller, M. R., & Papoutsakis, E. T., (1995). *Cell adhesion in animal cell culture: Physiological and fluid-mechanical implications*. Chapter 3, Cell adhesion: Fundamental and Biotechnological applications, Marcel Dekker, Inc.

Kotnik, T., Frey, W., Sack, M., Meglic, S. H., Peterka, M., and Miklavcic, D. (2015). Electroporation-based applications in biotechnology. Review, Cell Press, Trends in Biotechnology. 33:8, 480-488.

Kyoko A., Hiroyuki N. & Shoji T., (2004). *Protein Patterning with PDMS sieve*. Micro Electro Mechanical System, 17th IEEE International Conference on (MEMS), pp. 330-333.

Lea, R., Gorazd, P., & Damijan, M. (2013). *Electroporation of intercellular liposomes using nanosecond electric pulses – a theoretical study*, IEEE transaction on biomedical engineering, 60:9, 2624-2635.

Lodish, H., Berk, A., Zipursky, S. L., et al. (2000). *Cell-Cell Adhesion and Communication*. Molecular Cell Biology. 4th edition. New York: W. H. Freeman. Section 22.1.

Lodish, H., Berk, A., Zipursky, S. L., et al. (2000). *Cell-Cell Adhesion and Communication Molecular Cell Biology*. 4th edition. New York: W. H. Freeman, Section 22.1.

Lopez, G. P. et al. (1993). *Convenient method for patterning the adhesion of mammalian cells to surfaces using self-assembled monolayers of alkanethiolates on gold*. J Am Chem Soc, 115:5877-8.

Maksud, M. I., Yusof, M. S., & Abdul Jamil, M. M. (2013a). *A Study on Printed Multiple Solid Line by Combining Micro contact and Flexographic Printing Process for Microelectronic and Biomedical Applications*. International Journal of Integrated Engineering, 5(3), 36-39.

Maksud, M. I., Yusof, M. S., & Abdul Jamil, M. M. (2013b). *An Investigation of Parameter Effect on Micro contact Printing and Feasibility Study for Application in Microelectronic and Biomedical*. The 2013 Biomedical Engineering International Conference (BMEiCON-2013).

Marcus, S. N., Parul, S., Fuhrmann, B., Leipner, H. S. & Groth, T. (2011). *Cell Adhesion on Nanostructured Surface Designed by Nanosphere Lithography*. 10th International Workshop on *Biomedical Engineering*, 1 – 4, 5 – 7 October 2011.

Mark, J. J., Richard, G., Claude, N. & Richard, H. (1999). *In vivo gene delivery by electroporation*. Advanced drug delivery reviews, 35:1, 131-137.

Martin, A. S. & Richard, K. A. (2001). *Intergrin and cell proliferation: regulation of cyclin-dependent kinases via cytoplasmic signalling pathways*. Journal of cell science 114, Signal transduction and cellular organization, 2553-2560.

Marty, M., Sersa, G., Garbay, J. R., Gehl, J., Collins, C. G., Snoj, M., Billard, V., Geertsen, P. F., Larkin, J. O., Miklavcic, D., Pavlovic, I., Paulin-Kosir, S. M., Cemazar, M., Morsli, N., Soden, D. M., Rudolf, Z., Robert, C., O'Sullivan G. C., & Mir, L. M. (2006). Electrochemotherapy – An easy, highly effective and safe treatment of cutaneous and subcutaneous metastases: Result of ESOPE (European Standard Operating Procedures of Electrochemotherapy) study. ELSEVIER, EJC supplements 4, 3-13.

Merritt, M. V., Mrksich, M. & Whitesides, G. M. (1997). *Using Self-assembled monolayers too study the interactions of man-made materials proteins. Principles of Tissue Engineering*, Austin: R. G. Landes Co., 211-23.

Michael M., Adam J. C., et al. (2015). *Wound Healing and Repair*. Plastic surgery, Medscape. http://emedicine.medscape.com/article/1298129-overview [Access: 15/2/17]

Michael, W. K., (2016). *Extracellular Matrix*. http://themedicalbiochemistrypage.org/extracellularmatrix.php, 1996–2016 themedicalbiochemistrypage.org, LLC. [Access; 12/08/2016]

Miklavcic, D. & Towhidi, L. (2010). *Numerical study of the electroporation pulse shape effect on molecular uptake of biological cells*. Radiology and Oncology, 44(1), 34–41.

Min-Ji, K., Taeyoon, K. & Young-Ho, C. (2011). *A cell electroporation and viability monitoring chip using a single channel with multiple electric field zones*. 2011 16th International Solid-State Sensors, Actuators and Microsystems Conference (TRANSDUCERS), IEEE.

Mrksich, M. & Whitesides, G.M. (1995a). *Patterning self-assembled monolayers using micro contact printing: a new technology for biosensor*. Trends Biotech, 13:228-35.

Mrksich, M. & Whitesides, G.M. (1996b). *Using Self-assembled monolayers too study the interactions of man-made materials proteins and cells*. Ann Rev Biophysics Biomol Struct, 25:55-78.

Mrksich, M. et al., (1996). *Controlling cell attachment on countered surfaces with self-assembled monolayers of alkanethiolates on gold*. Proc Natl Acad Sci USA, 9310775-8.

Mrksich, M., Dike, L. E., Tien, J., Ingber, D. E. & Whitesides, G. M. (1997). *Using micro contact printing to pattern the attachment of mammalian cells to self-assembled monolayers of alkanethiolates on transparent films of gold and silver*. Experimental cell research, 235:305 – 313.

Nancy, L. H. & James J. T., (1995). *Mechanical Properties of the Extracellular Matrix Influence Fibronectin Fibril Assembly in Vitro*. Experimental Cell Research 217, 109-117.

Narang, V., Wong, S. Y., Leong, S. R., Abastado, J. P., & Gouaillard, A. (2011). *Comparing Mathematical Models of Cell Adhesion in Tumors*. Defense Science Research Conference and Expo (DSR), Singapore, 3 – 5 August 2011, 1-4.

Nobuyuki, T., Hiroki, O., Kazuhiro, F., Masayuki, Y. & Teruo, O. (2013). *Multiple micro contact printing of extracellular matrix with fine alignment micro nanomechatronics and human science (MHS)*. International symposium on japan.

Ostuni, E., Whitesides, G. M., Ingber, D. E. & Chen, C. S. (2009). *Using Self-assembled monolayers too pattern ECM proteins and cells on substrates*. Methods Mol. Biol., 552,183-194.

Paula, A. G., John, H. R. & Rafael, V. D. (2011). *Electrical conductivity changes during irreversible electroporation treatment of brain cancer*. 2011 Annual International Conference of Engineering in Medicine and Biology Society, EMBC, IEEE, 30 Aug.-3 Sept.

Quist, A. P., Pavlovic, E. & Oscarsson, S. (2005). *Recent advances in micro contact printing*. Review, Anal Bioanal Chem, 381: 591-600.

Rachel, K. S., Penelope, A. L. & Paul S. W. (2004). *Patterning self-assembled monolayers*. Review, progress in surface science, 75:1-68.

Rahul, B. & Anil, M. (2010). *Surface patterning using self-assembled monolayers (SAMs)*. Biomaterials, ACS symposium series, 1054:4, 65-107.

Ravi, S. K., Shuichi, T., Emanuele, O., Donald, E. I., & George, M. W., (1999). *Patterning proteins and cells using soft lithography*. Biomaterials 20, 2363-2376.

Rinaldi, R., Pompa, P. P., Maruccio, G., Biasco, A., Visconti, P., Pisignano, D., Blasi, L., Sgarbi, N., Krebs, B. & Cingolani, R. (2004). *Self-assembling of proteins and enzymes at nanoscale for biodevice applications*. IEE proceedings, Proc-Nanobiotechnol, 151(3), June 2004.

Rodamporn, S. (2012). *Optimal Parameters of Electroporation for Gene and Tissue*. The 2011 Biomedical Engineering International Conference (BMEiCON-2011), Chiang Mai, Thailand, 29 - 31 Jan 2012, 279-282.

Santra, T.S., Pen-cheng, W., & Gang, F., (2013). *Electroporation based drug delivery and its applications*. Chapter 3, Advanced in micro/nano electromechanical system and fabrication technologies, intech, 38.

Scholl, M., Sprossler, C., Denyer, M., Krause, M., Nakajima, K., Maelicke, A., Knoll, W., & Offenhausser, A. (2000). *Ordered networks of rat hippocampal neurons attached to silicon oxide surfaces*. Journal of Neuroscience Methods, 104 (1), 65-75.

Sersa, G., Cemazar, M., & Snoj, M. (2009). *Electrochemotherapy of tumours*. Current Oncology. Updates and development in Oncology, 16:2, 34-35.

Singhvi, R., et al. (1994). *Engineering cell shape and function*. Science 1994; 264:696-8.

Singhvi, R., Kumar, A., Lopez, G. P., Stephanopoulos, G. N., Wang, D. I., Whitesides, G. M. & Ingber, D. E. (1994). *Engineering Cell and Function*. NCBI, Science 1994 Apr 29; 264 (5159):696-8.

Stankevič, V. et al. (2013). *Electroporation system generating wide range square-wave pulses for biological applications*. IEEE Biomedical Circuits and Systems Conference (BioCAS), Rotterdam, 33-36.

Straughan, D. W., Fentem, J. H. & Balls, M. (1997). *Replacement alternative and complementary in vitro methods in pharmaceutical research*. In vitro methods in pharmaceutical research, academic press, medical, 467.

Stroncek, J. D. & Reichert, W. M. (2008*). Overview of Wound Healing in Different Tissue Types*. In: Reichert, W.M., editor. Indwelling Neural Implants: Strategies for Contending with the In Vivo Environment. Boca Raton (FL): CRC Press/Taylor & Francis; 2008; 1.

Sundararajan, R. (2014). *Electroporation-Based Therapies for Cancer: From Basics to Clinical Applications*. Elsevier, Aug 28, Medical, 352.

Sundararajan, R., Rajendran, R., Shahid, S. S., Santosh, D.K., Radhakrishnan, S., Priyadarshan, K., Varsha, S., Kumar, U. V., Ramachandran R. & Sankaranarayanan, K. (2011). *Effect of irreversible electroporation on cancer cells*. IEEE.

Sundararajan, R., Salameh, T., Camarillo, I. & Campana, L. (2011). *Effective use of electrical pulses on cancer cells to control proliferation*. IEEE, 14th International Symposium on Electrets (ISE), 28-31 Aug.

Sundararajan, R., Xiao, F., Otto, K. & Camarillo, I. G. (2012). *The Dielectric properties of electroporated human breast cancer cells*. IEEE 10th international Conference on the Properties and applications of dielectric materials, July 24-28, Bangalore, India.

Sundararajan, R., Xiao, F., Salameh, T., Reece, L. M., Leary, J. F., Otto, K., Camarillo, I. G. & Campana, L. G. (2012). *Effective Proliferation control of human cancer cells using electrical pulses.* IEEE Transactions on dielectrics and electrical insulation, 19:6, 2225-2236.

Sutherland, J., Smith, A. G., Egan, A. C., Denyer, M. C. & Britland, S. T. (2005). *Control of Human Skin Cell Adhesion, Proliferation and Migration by Microengineered substrata: transposition from solid phase to clinically relevant materials*. Proceedings of The 3th Annual International IEEE EMBS Special Topic, *Conference on Micro technologies in Medicine and Biology*, Kahuku, Oahu, Hawaii.

Thery, M. & Piel, M. (2009). *Adhesive Micro pattern for cells: a micro contact printing protocol*. Cold Spring Harb Protocol, 2009; 7.

Thomas, C. A., Springer, P. A., Loeb, G. E., Berwald, N. Y. & Okun, L. M., (1972). *A miniature microelectrode array to monitor the bioelectric activity of cultured cells*. Exp Cell Res 1972; 74:61-6.

Tobias, K. & Bart, J. R. (2010). *Stamps, inks and substrates: polymers in micro contact printing*. Polym. Chem, 1, Issue 4, 371-387.

Uhlén, et al. (2015). *Tissue-based map of the human proteome.* Science PubMed: 25613900 DOI: 10.1126/science.1260419.

Van Der Valk, J., Mellor, D., Brands, R., Fischer, R., Gruber, F., Gstraunthaler, G., Hellebrekers, L., Hyllner, J., Jonker, F.H., Prieto, P., Thalen, M. & Baumans, V. (2004). *The Humane Collection of Fetal bovine serum and Possibilities for serum-free cell and tissue culture*. Elsevier, Toxicology in vitro, 18:1-12.

Vera Tizalt, A. L., Garay Jimenez, L. I. & Rodriguez Cuevas, S. A. (2013). *3D Model and Simulation of Electroporation Application on Health and Tumoral Breast Tissue*. *10th International Conference on Electrical Engineering*, Computing Science and automatic control (CCE), Mexico City, Mexico.

Weaver, J. C. (2000). *Electroporation of Cells and Tissues*. IEEE Transactions on Plasma Science, 28.

Xia, Y. & Whitesides, G. M. (1998). *Soft Lithography*. Angew. Chemie Int. Ed, 37(5); 550-575.

Xiao, J. L., Lu, D., Chiu Y. T. & Lee, C. H. (2013). *Cell Migration Guidance by using Optical Micro patterns.* IEEE, *Conference on Laser and Electro-Optics*- Pacific Rim (CLEO-PR).

Yin, H. B., Brown, T., Wilkinson, J. S., Eason, R. W. & Melvin, T. (2004). *Submicron patterning of DNA oligonucleotides on silicon*. Nucl Acids Res, 32 (14): e118, 1-7.

Yoshiro, I. (1999). *Surface micro patterning to regulate cell functions*. Elsevier, Biomaterials 20; 2333-2342.

Zaltum, M. A. M., Adon, M. N. & Abdul Jamil, M. M. (2013). The biomedical engineering international conference (BMEiCON-2013).

Zhu, B., Zhang, Q., Lu, Q., Xu, Y., Yin, J., Hu, J. & Wang, Z. (2004). *Nanotopographical guidance of C6 glioma cell alignment and oriented growth*. Elsevier, Biomaterials, 25; 4215-4223.